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Is Long-Distance Bird Flight Equivalent to a High-Energy Fast? Body Composition Changes in Freely Migrating and Captive Fasting Great Knots

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ABSTRACT

We studied changes in body composition in great knots, *Calidris tenuirostris*, before and after a migratory flight of 5,400 km from northwest Australia to eastern China. We also took premigratory birds into captivity and fasted them down to their equivalent arrival mass after migration to compare organ changes and nutrient use in a low-energy-turnover fast with a high-energy-turnover fast (migratory flight). Migrated birds were as economical as any fasting animal measured yet at conserving protein: their estimated relative protein contribution (RPC) to the energy used was 4.0%. Fasted birds had an estimated RPC of 6.8% and, consequently, a much lower lean mass and higher fat content for an equivalent body mass than migrated birds. Lean tissue was catabolized from most organs in both groups, except the brain. Furthermore, a principal components biplot showed that individuals were grouped primarily on the basis of overall organ fat or lean tissue content rather than by the size of specific organs. This indicates that organ changes during migratory flight are similar to those of a low-energy fast, although the length of the fast in this study probably accentuated organ reductions in some functional groups. Whether the metabolic characteristics of a flying migratory fast follow the three-phase model described in many inactive fasting animals is unclear. We have some evidence for skeletal fat being catabolized without phase 3 of a fast having been reached.

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Introduction

Long-distance migration flight for birds is an intense period of starvation when all nutrients used to maintain metabolic processes are drawn from stored deposits. In anticipation of migratory flight, birds deposit substantial fat stores (up to or even exceeding 50% of total body mass; Jehl 1997; Piersma and Gill 1998) as well as lean tissue in various organs (e.g., Fry et al. 1972; McLandress and Raveling 1981; Marsh 1984; Evans et al. 1992; Piersma et al. 1999*b*). Similar changes are found in anticipation of natural fasts in inactive birds such as Antarctic penguins (Cherel et al. 1993) and Arctic geese and ducks (Raveling 1979; Parker and Holm 1990). During long-term fasts and long-distance flight, body mass declines by up to half (even more in some penguins), with both fat and protein being catabolized (Cherel et al. 1988; Battley et al. 2000*a*).

The pattern of nutrient use during inactive starvation is well documented (Cherel et al. 1988; Boismenu et al. 1992) and consists of three phases: phase 1, a short phase of high body mass loss and increasing reliance on fat for fuel; phase 2, a long phase of low protein and high fat catabolism (which may last for up to 4 mo in king penguin [*Aptenodytes fosteri*] chicks); and phase 3, a period of accelerating body mass loss and increasing protein breakdown that can lead to nutrient exhaustion. How similar in this respect is flight to fasting? This is an important question because fasting is often used to simulate the changes that occur in migrating birds (Klaassen and Biebach 1994; Hume and Biebach 1996; Biebach 1998; Karasov and Pinshow 1998, 2000). Contrary to earlier views (Odum et al. 1964), protein is not used in flight only in emergency situations after fat is depleted but is probably continuously catabolized, most likely for the maintenance of citric acid–cycle intermediates (for fatty acid oxidation) and for gluconeogenesis (Jenni and Jenni-Eiermann 1998). Migrating passerines arriving in Italy on migration provide evidence of accelerated protein breakdown at low fat levels (Jenni et al. 2000). But while flight

and fasting share certain features, a major difference is the rate of energy turnover, which is several times higher in flying birds. Given the constraints in fuel supply experienced by flying birds, especially for long-distance migrants (Jenni and Jenni-Eiermann 1998), the pattern of body mass change and resulting body composition may differ between flown and fasted birds. This has not been investigated in detail, although several authors (cited above) have recently used short-duration fasts to simulate migratory starvation when studying organ sizes. The results of these studies, and of analyses of wild migrating birds (Åkesson et al. 1992; Battley et al. 2000*a*), all indicate that substantial changes in organ lean masses occur in migrating or captive fasting birds. Birds flying for several days could be limited in their ability to spare protein during flight, given constraints in energy supply from lipids and the long duration of flight, but the few data on migrating birds indicate that they are extremely effective at minimizing the energetic contribution of protein (Jenni and Jenni-Eiermann 1998). Likewise, the only data on animals fasting under different energy expenditure regimes (thrush nightingales, *Luscinia luscinia*, with a twofold difference in metabolic requirements [Klaassen and Biebach 1994]; hedgehogs, *Erinaceus europaeus*, during hypothermia with a 2.5-fold range in total energy expenditure [Cherel et al. 1995]) showed similar protein contributions to the overall energy use.

We investigated energy use and body composition changes in a long-distance migrant shorebird, the great knot, *Calidris tenuirostris*. Our aim was to determine whether changes in organ size differ between wild birds undergoing a high-energy-turnover fast (a migratory flight of 5,400 km lasting 4 d) and captive birds undergoing a low-energy-turnover fast (a period of starvation down to the equivalent body mass at arrival of migrating birds). Great knots were caught before departure on northward migration in northwest Australia and after arrival in China. Some birds caught before migration were taken into captivity and fasted. We could, therefore, compare the body condition of both flown and fasted birds with predeparture condition. We expected to find dramatic changes in body composition in the migrated birds, which had just completed one of the longest known flights in the world (see Battley et al. [2000*a*] for evidence for this flight). The distance between Roebuck Bay, northwest Australia, and the Yangtze River, China, is 5,420 km, and band recoveries, timing of departures from Australia and arrivals in China, and a lack of large numbers of birds anywhere in between on migration all indicate that most birds perform this flight in one go (Battley et al. 2000*a*). While the majority of great knots appear to head for the west coast of South Korea, Chongming Island, China, is a known arrival site for migrating great knots.

In the fasted group of great knots, the large change in body mass during migration was simulated without the hard work of flight, which could provide insight into the mechanisms behind any organ changes. We predicted that if physical work during flight increased tissue catabolism, then the fasted birds would use proportionately less tissue from the exercise organs used in sustained flight than the migrated birds. For reasons of mechanical efficiency, it could also be advantageous for a migrant to reduce the size of exercise organs during flight (Pennycuick 1998). The flown birds had an energy turnover rate almost five times that of the fasted group, enabling us to compare the relative fuel contributions in situations of very different levels of energy expenditure.

Material and Methods

Samples

The three main groups of birds in this study represent (1) birds caught as close to migration as was possible in Australia and killed immediately (predeparture group, $n = 10$); (2) birds caught before migration in Australia, transported live to The Netherlands Institute for Sea Research (NIOZ), and fasted down to an estimated arrival mass (fasted group, $n = 8$, three of which were killed for body composition analyses when they had reached published [Barter et al. 1997] arrival masses, taking into account body size variation as expressed by wing length); and (3) birds caught in China soon after having arrived on migration from Australia (postmigration group, $n = 10$; birds were apparently passing through the study site and not staying, so birds were probably caught between a few hours and a day or two after arrival). The fasted birds were force-fed a limited amount of food (kitten pellets and Trouvit pellets) until their arrival in The Netherlands and had free access to fresh water while fasting. The aim was to keep the birds healthy (the combination of heat and captivity caused some birds to temporarily suffer leg cramp) and to ensure they did not lose too much mass before metabolic measurements could be made during their fast (P. F. Battley, A. Dekinga, M. W. Dietz, T. Piersma, S. Tang, and K. Hulsman, unpublished manuscript). They were weighed three times between capture and transport (5-d period) but daily after arrival at the NIOZ. At the NIOZ, they were kept at 17°–21°C under a 12L : 12D light-dark cycle. Only three of the eight birds were killed at the end of the fast. The rest were refed to form a captive flock for ongoing annual cycle studies.

The predeparture and fasted groups were caught by cannon net on the afternoon of March 21, 1998, at Roebuck Bay, northwest Australia (18°00′S, 122°22′E). First migratory departures were observed on March 27, 1998, but tidal heights made catching impossible after March 21. Hunters using clap nets and decoys toward the southeastern end of Chongming Island, Yangtze River estuary, China (30°48′N, 121°27′E), caught postmigration birds between April 1 and April 9, 1998. A fourth group of great knots was caught earlier during fueling at Roebuck Bay in 1998 but will not be analyzed in as much detail as the other groups in this article. There were two birds caught

by zap net on February 20, 1998, and 10 birds caught by cannon net on March 6, 1998 (fueling group).

For the birds that were killed for body composition analysis, a multivariate estimate of structural size was used to test for a difference in mean size between the predeparture and postmigration birds (based on a principal components analysis of 11 external and internal structural measurements: bill, total head, tarsus, tarsus + midtoe, wing length, plus six measurements of the sternum). These groups did not differ in body size (*t*-test, $t_{18} = -0.0369$, $P = 0.971$). Only external measurements are available for birds that were not killed. Birds killed on March 21 were not larger than other birds in the catch in any measure (the only significant difference was that wing length was less: 187.9 ± 3.6 vs. 191.6 ± 3.7 mm, $t_{39} = 2.448$, $P = 0.019$). The birds taken into captivity for fasting did not differ significantly from the predeparture birds in wing length ($t_{16} = 1.968$, $P = 0.069$), tarsus ($t_{16} = 1.748$, $P = 0.100$), tarsus + midtoe ($t_{16} = -0.723$, $P = 0.493$), or total head length ($t_{16} = 1.123$, $P = 0.278$). (A significant difference in bill length probably resulted from bill lengths of the captive birds not being measured until after the birds had lived in captivity for some while [$t_{16} = 2.122$, $P = 0.050$].) We did not inadvertently collect the largest birds out of the catches, and birds in the different samples do not differ in body size.

For body composition analyses (see next section), masses were corrected for structural size variation between individuals based on the multivariate size estimate mentioned above. Organs were standardized by dividing organ mass (or measure) by the ratio of the size estimate of that individual to the mean size estimate for all birds. Packard and Boardman (1999) show that this approach can introduce errors into the analysis and advocate ANCOVA to correctly account for body size differences. In our study, we used randomization tests rather than conventional analyses, and size-corrected data were more practical to use. In any case, the absolute variation between individuals in size was small: the range of size estimates was only 6% of the mean size estimate. A quick comparison between an ANOVA, using size-corrected data, and an ANCOVA, using the size estimate as the covariate, indicated that in this study, results are not compromised by using size-corrected data. Comparisons between lean mass of 15 organs in the three groups in this study showed that probability differences between the methods were very small and never affected overall significance. ANCOVA detected size effects in only three of the 15 comparisons, indicating that size influences were generally low.

Total body mass comparisons involving all birds were not corrected for size because a limited number of external measurements were available for the birds of the fasted group that were not killed. In body composition analyses involving total body mass, however, body mass was corrected for body size differences for consistency with organ analyses. Sexes were combined for body mass comparisons because sexes of the living fasted birds are unknown.

Total body masses for birds cannon netted in Australia were corrected for water loss between capture and weighing by between factors of 1.004 and 1.056, calculated from a mass loss experiment performed on 10 great knots from March 21.

Most birds in Australia were killed on the evening of the day they were caught. Five birds were killed the next morning after respirometry. One bird died in a respiratory chamber because of a pump malfunction. In China, only two birds were killed on the day of capture. The rest were used in respirometry and killed the next morning (six birds) or the morning after that (two birds were held in captivity for an extra day to allow respirometry and fed on fresh fish through the day). The respirometry procedures involved placing two birds in 9- or 11-L Perspex containers through which dried air flowed in the evening (or equivalent evening for the captive birds). The concentration of O_2 (and in the captive birds, CO_2) was measured alternately for the two birds through the night, switching to reference after every hour. In the morning, birds were removed and killed by cervical dislocation or returned to their enclosure. Temperatures during measurements were on average 30.8°C in Australia (ambient), 23.3°C in China (heated), and 24°C in captivity (heated). More details on the respirometry protocols are provided in P. F. Battley, A. Dekinga, M. W. Dietz, T. Piersma, S. Tang, and K. Hulsman (unpublished manuscript). Birds from the three key groups showed virtually no body molt, so nutritional demands of molt will not affect relative nutrient use in our analyses.

Body Composition Analyses

After birds were killed by cervical dislocation, the left pectoral muscle was removed and frozen at −60°C. Carcasses were sealed in plastic bags and frozen at −20°C until dissection in December 1998. Birds were transported on dry ice to the NIOZ, where they were dissected by P. F. Battley. After plucking, the following organs were dissected out: flight muscles (supracoracoideus and pectoralis), leg muscles, heart, lungs, intestine, liver, stomach, spleen, kidneys, salt glands, brain, abdominal fat (a discrete deposit in the abdominal cavity, including fat adhering to the stomach), skin (with associated subcutaneous fat layer), tibia, and lower legs. The remainder of the carcass after removing all organs is referred to as the "rest" and comprises mainly the skeleton and adhering muscle. Certain organs or parts of organs were retained for further work: one tibia, one lower leg, a section of leg muscle, three sections of intestine, half the liver, and half the remaining pectoral muscle. These parts were weighed fresh (±0.01 g), and their contributions to dry and fat-free masses were estimated via the equivalent masses of the remainder of the organs. Organs were dried to constant mass at 60°C. Lipids were extracted from the tissues by petroleum ether extraction (40°–60°C, soxhlet apparatus). By subtraction, fat and lean dry tissue contents of the organs were calculated. We use lean mass to refer to fat-free dry mass

Table 1: Individual body masses (g) of great knots of different groups

	Fueling	Predeparture	Start of Fast	End of Fast	Postmigration
Individual:					
1	178	217	214	125	118
2	184	220	217	127	118
3	195	232	219	127	121
4	201	234	222	129	121
5	202	238	235	130	122
6	202	240	237	131	123
7	204	244	237	132	125
8	208	253	239	133	127
9	209	256	…	…	137
10	210	259	…	…	137
11	219	…	…	…	…
12	242	…	…	…	…
Average	204.5	239.4	227.5	129.3	124.9

Note. Fueling birds were caught on February 20 and March 6, 1998, predeparture birds were caught on March 21, 1998, birds were taken into captivity for fasting on March 21, 1998, postmigration birds were caught in China from April 1 to April 9, 1998, and the fasted group was taken at the end of their individual fasts. Body masses of the birds caught in Australia were corrected for water loss between capture and weighing (see "Material and Methods"). Birds are presented in order of increasing body mass.

rather than fat-free fresh or wet mass. Fat-free dry mass is the most accurate unit for comparison because water content of individual organs is difficult to measure accurately when making detailed dissections. For the salt glands, only dry mass was determined. Two whole organs were retained for further study—the heart and stomach. Fresh mass was determined directly for these, but for total fat mass calculations, the fat content of these organs was estimated, based on data from nine great knots that were accidentally killed during catching attempts in 1996. Hearts and stomachs generally contain very little fat in a wide range of shorebirds (T. Piersma, unpublished data). The rest of the carcass was burned at 600°C to determine the ash content. Spleen masses were measured to ± 0.0001 g.

Statistical Analyses

Means are given ± 1 SD. Student's *t*-tests were used to determine whether body masses differed between predeparture and fasting groups at capture and between fasted and flown birds. ANOVA was used to test whether individuals varied in the rate of body mass loss during fasting. Because sample sizes were small, randomization tests were used to test for significant differences between organs in different groups of birds, using the RT program of Manly (1997). For each organ comparison, we tested for an overall difference across the three groups by using the Rtanova program, calculating the *F* statistic from the data, randomizing the data 10,000 times, generating the *F* statistic each time, and comparing the observed statistic with the distribution of randomly generated ones. The probability value represents the chance that the observed patterns in the data could have resulted from random assortment of the values. Because we were interested in how similar or different the flown and fasted groups were, we then performed a two-sample randomization on these two groups using the Rt2samp program. The mean difference between the groups was calculated, the samples randomized 10,000 times, and the resulting differences calculated. A two-tailed probability (probability of a value as far or farther from zero) was calculated. Because two comparisons were performed on the same data, the comparison-wise error rate (level of significance) for both comparisons was set at $0.05/2 = 0.025$. As the sexes of great knots are monomorphic, we could not select birds according to sex and ended up with male-biased samples (seven of 10 in predeparture birds in Australia, six of 10 postmigration birds in China, and all three birds killed after fasting). The females tended to be the heavier, fatter birds in Australia but the leaner birds in China. Our samples are too small to meaningfully test for sex differences, so we analyzed both sexes together. One apparently nutrient-stressed female in China (discussed further in "Results") was not included in the main body composition analyses.

The principal components biplot was used to assess how correlated the changes of individual organs were in relation to changes in body composition (Gabriel 1971; see Piersma et al. 1996 for an example). This method plots the position of individual birds on the first two axes of a principal components analysis, as well as the vectors based on the correlation matrix of the organs. The length and proximity of the vectors reveal the correlations between different organs: technically, they are the eigenvalue loadings of the principal components analysis,

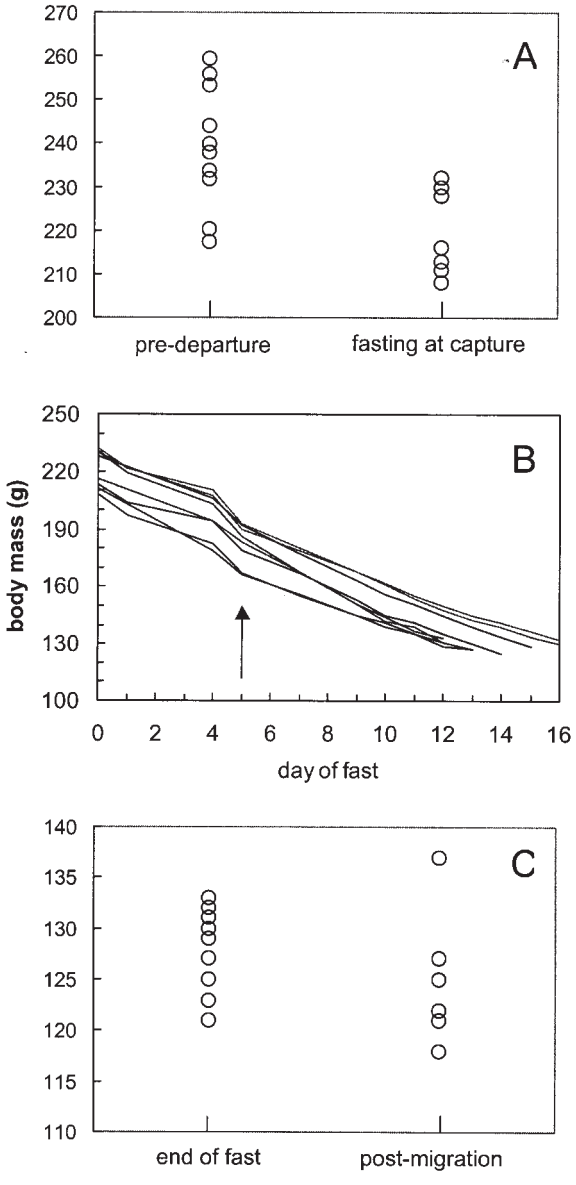

Figure 1. Body masses of the three groups of great knots in this study. *A*, Mass at capture on March 21 of predeparture and experimentally fasted birds. *B*, Rate of mass loss for eight great knots during fasting in captivity. The arrow (day 5) shows the start of the experimental fast (before this, birds were fed while transported to The Netherlands). The fast finished when birds reached an estimated arrival mass, and they were fed (five) or killed (three). *C*, Mass of birds recently arrived on migration in China (postmigration) and birds at the end of their fast (some points overlap in the plot). There was a delay before weighing during capture on March 21, so we estimated their fresh masses on the basis of a mass-loss experiment performed on 10 great knots from the same catch. The delay between capture and weighing was 0.5–7 h, and fresh masses were estimated as fresh mass = time since capture (h) × 0.0079 × mass at first weighing.

so they visually provide the interpretation of the axes. The actual loadings are provided in the appendix. More technical details are provided by Piersma et al. (1996, 1999*b*), and further details on interpretation are provided in the caption to Figure 3. The biplot procedure was carried out in SYSTAT 8.0 (SPSS 1998) and performed on untransformed (but size-corrected) values for fat masses and lean tissue masses.

Results

Mass Loss during Fasting and Flight

Individual and mean masses of all the groups are provided in Table 1. In Australia, the birds killed on March 21, 1998, were heavier on average than the birds taken into captivity, though not significantly (Fig. 1*A*; sexes combined: $t_{16} = 1.962$, $P = 0.067$). This resulted from the heaviest birds suffering the most from heat stress and being killed rather than taken into captivity. Mean masses were 239.4 ± 14.3 and 227.5 ± 10.2 g for predeparture and prefasting birds, respectively (note that average departure mass is over 240 g; Higgins and Davies 1996).

After arrival in The Netherlands, mass loss of the experimental birds during their fast down to estimated arrival mass was rather linear (Fig. 1*B*), with an average individual loss of 6.3 g d^{-1} (range 4.7–8.6 g d^{-1}). Differences between individuals in the rate of mass loss were significant (ANOVA, day × individual, $F_{52,7} = 5.392$, $P < 0.001$), even for the six birds whose rates of mass loss lay between only 5.8 and 6.6 g (though only just; ANOVA, day × individual, $F_{42,5} = 2{,}446$, $P = 0.049$). After fasting, the experimental birds weighed on average 129.3 ± 2.8 g, which was not significantly different from the mass of postmigration birds in China (124.9 ± 7.0 g; $t_{16} = 1.66$, $P = 0.116$; Fig. 1*C*).

Body Composition

Before departure, most fat was contained in discrete deposits, particularly the subcutaneous (skin) and abdominal deposits (Table 2). Most organs contained only small amounts of fat, the exceptions being the flight muscles, leg muscles, intestine (much of which was a separate layer adhering to the outside of the intestine), and the rest of the carcass. Across the three main groups, there were significant differences in the amount of fat in every organ except the brain.

The only organs that were not significantly different in fat content between the postmigration and fasted birds were the flight muscles, lungs, and brain. In all the other organs, in all cases, the fasted group retained more fat. Three organs dropped in fat content only in the migrated birds: the liver, the tibia, and the lower leg. The experimentally fasted birds had over twice the fat content of the postmigration birds, especially in subcutaneous and abdominal deposits (Table 2).

In both the postmigration and fasted groups, total lean tissue was also lower than in predeparture condition (Table 3). The brain and two leg bone components were the only organs that

Table 2: Distribution of fat (g) in bodies of great knots before and after a migratory flight of 5,400 km and after a fast in captivity of approximately 2 wk

Body Part	Predeparture ($n = 10$)	Postmigration ($n = 9$)	Experimentally Fasted ($n = 3$)	Overall Difference		Postmigration vs. Fasted P
				F	P	
Flight muscles	2.359 ± .440	1.044 ± .268	1.438 ± .433	29.72	**.0001**	.0883
Leg muscles	1.275 ± .384	.362 ± .110	.824 ± .090	26.08	**.0001**	**.0001**
Lungs	.084 ± .027	.027 ± .017	.030 ± .030	15.82	**.0002**	.7828
Skin	59.930 ± 3.913	1.940 ± 1.205	11.077 ± 3.451	938.64	**.0001**	**.0001**
Abdomen	12.475 ± 3.142	.698 ± 1.127	3.246 ± 1.806	62.24	**.0001**	**.0187**
Intestine	3.680 ± .466	.431 ± .194	.843 ± .303	211.841	**.0001**	**.0133**
Liver	.302 ± .100	.073 ± .026	.317 ± .128	21.24	**.0002**	**.0001**
Kidney	.197 ± .062	.054 ± .016	.098 ± .037	24.10	**.0001**	**.0122**
Brain	.105 ± .017	.095 ± .012	.101 ± .002	1.23	.3083	.3985
Rest	8.421 ± 1.680	6.233 ± 1.404	9.659 ± .632	8.17	**.0026**	**.0001**
Tibia	.501 ± .097	.243 ± .045	.418 ± .078	26.95	**.0001**	**.0001**
Lower leg	.159 ± .068	.054 ± .055	.168 ± .060	8.03	**.0030**	**.0087**
Total fat[a]	89.847 ± 6.829	11.547 ± 3.791	28.429 ± 4.761	500.76	**.0001**	**.0001**
Deposits[b]	76.085 ± 5.551	3.069 ± 2.287	15.166 ± 5.362	682.24	**.0001**	**.0001**
Organs[c]	13.762 ± 2.175	8.479 ± 1.735	13.263 ± .872	19.87	**.0001**	**.0001**

Note. Values presented are mean ± SD. P values are from randomization tests with the comparison-wise error rate for significance set at 0.025. Significant results are in bold.

[a] Includes estimated fat mass of heart and stomach, based on the relationship between fresh mass and fat mass for a sample of accidentally killed great knots from northwest Australia.

[b] Fat contained under the skin, in the abdominal cavity, and in and around the intestine.

[c] Total fat minus the deposits, including the carcass.

did not differ in lean tissue mass between the groups (fresh heart mass was also not significant). In every organ in which there was a significant difference between the lean tissue of flown and fasted birds, the fasted birds' lean tissue was lower. Nutritional organs were especially reduced, to 68.4% of the equivalent predeparture mass in postmigration birds and only to 39.0% in the fasted birds. For exercise organs, the respective percentages were 80.5% and 68.4%. Expressed another way, postmigration birds had 1.14 times the total lean tissue of the fasted birds, 1.09 times the exercise organ tissue, and 1.75 times the nutritional organ matter. In both relative and absolute terms, the rest of the carcass, flight muscles, skin, and intestine (and stomach fresh mass) contributed most to the reduction in the fasted birds. These differences were also apparent in the length of the intestine, which was 14.6% shorter than predeparture in the postmigration birds and 24.0% shorter in the fasted birds. Ash content of the rest of the carcass was not lower in the postmigration birds but was reduced in the fasted group (though not significantly, probably because of the small sample size: $P = 0.3492$ from a randomization test between predeparture and fasted birds; Table 3).

Correlations between Organs

The biplots (Fig. 2) reveal both correlated changes in organ masses and whether different groups of individuals can be distinguished by their organ masses. In fat content (Fig. 2*A*), the organs were all fairly highly correlated with each other, with no clear subsets of organs. Within this organ sector some organs were, however, extremely highly correlated with each other (skin and intestine, abdominal fat and liver), and the brain (with a short vector) was only weakly correlated with other organs. Groups of individuals were clearly separated, with only one fueling bird falling within the predeparture groups. Much of the discrimination among birds was provided by axis 1, which reflected overall organ fat contents (indicated by the directions of the organ vectors). Individual organs provided little difference between the groups apart from between the fasted and flown birds (axis 2).

Changes in organ lean tissue were more disparate (Fig. 2*B*). Some organs (brain, tibia, lower leg, and spleen) were not, or only weakly, correlated with the majority of the organs. The remaining organs (leg muscles, flight muscles, salt glands, kidneys, skin, lungs rest, intestine, and liver) formed a group in the mid-right-hand side of the plot. Again, discrimination between individuals in groups was almost entirely found in axis 1, related to overall organ lean masses. Discrimination between organs was largely (apart from the brain) found on axis 2, but this did not provide any distinction between groups of individuals. Changes in lean tissue mass in great knots preparing

Table 3: Distribution of fat-free dry tissue (g) in bodies of great knots before and after a migratory flight of 5,400 km and after a fast in captivity of approximately 2 wk

Body Part	Predeparture ($n = 10$)	Postmigration ($n = 9$)	Experimentally Fasted ($n = 3$)	Overall Difference		Postmigration vs. Fasted P
				F	P	
Flight muscles	8.895 ± .700	7.094 ± .437	6.778 ± .185	30.87	**.0001**	.2630
Leg muscles	1.294 ± .102	1.157 ± .111	1.118 ± .123	5.22	**.0151**	.6063
Lungs	.830 ± .113	.714 ± .074	.401 ± .055	24.71	**.0001**	**.0039**
Skin	3.702 ± .453	2.169 ± .265	2.089 ± .459	44.17	**.0001**	.6920
Abdomen	.181 ± .052	.054 ± .074	.057 ± .006	12.18	**.0002**	.9616
Intestine	1.673 ± .299	.976 ± .172	.595 ± .149	32.84	**.0001**	**.0084**
Liver	1.578 ± .325	.944 ± .179	.625 ± .071	23.51	**.0001**	**.0183**
Kidney	.637 ± .090	.440 ± .073	.280 ± .039	29.29	**.0001**	**.0084**
Salt glands	.098 ± .020	.055 ± .010	.037 ± .006	26.92	**.0001**	**.0137**
Brain	.202 ± .013	.212 ± .019	.202 ± .008	1.21	.3142	.3707
Spleen	.019 ± .008	.011 ± .004	.007 ± .001	6.05	**.0103**	.1032
Rest	13.621 ± 1.046	12.257 ± .414	11.350 ± .653	12.32	**.0003**	**.0104**
Tibia	.653 ± .062	.621 ± .029	.597 ± .012	2.08	.1520	.1999
Lower leg	1.125 ± .116	1.082 ± .083	1.031 ± .101	1.10	.3555	.3823
Total fat-free dry matter[a]	37.363 ± 2.885	30.141 ± 1.263	26.512 ± 1.131	41.09	**.0001**	**.0039**
Exercise organs[b]	10.334 ± .796	8.326 ± .520	7.630 ± .164	32.43	**.0001**	.0519
Nutritional organs[c]	6.134 ± 1.118	4.196 ± .594	2.393 ± .332	25.55	**.0001**	**.0039**
Heart fresh mass	2.351 ± .437	2.000 ± .382	1.739 ± .309	3.39	.0618	.3067
Stomach fresh mass	7.942 ± 2.086	6.476 ± 1.513	3.133 ± .346	9.42	**.0009**	**.0039**
Rest ash[d]	3.394 ± .733	3.420 ± .359	2.798 ± .785	1.27	.3492	.1100
Intestine length (cm)	57.794 ± 3.916	49.372 ± 4.204	43.914 ± 6.379	15.52	**.0003**	.1097

Note. Values presented are mean ± SD. P values are from randomization tests with the comparison-wise error rate for significance set at 0.025. Significant results are in bold.

[a] Includes estimated fat-free dry mass of heart and stomach, based on the relationship between fresh mass and fat mass for a sample of accidentally killed great knots from northwest Australia.

[b] Combined masses of flight muscles, heart, and lungs.

[c] Combined masses of stomach, intestine, liver, and kidneys. Because the stomach and heart were not processed in detail, fresh mass is given for comparison.

[d] Mass of the remainder of the carcass after incineration ($n = 6$ for predeparture).

for and undertaking migration, and fasting in captivity, involved so many organs (Table 3) that there were no clear differences in groups of birds that can be related to specific organs. This does not contradict Table 3, which showed significant differences between the key groups in most organs, including exercise and nutritional organs; rather, it stresses how ubiquitous the organ changes are in the body. An apparently nutrient-stressed female from China (Fig. 2, *filled circle*) clearly fell well away from the other Chinese birds, justifying its omission from earlier analyses.

Changes in Nutrient Composition during Fueling and Fasting

We lacked sufficient samples during fueling to accurately assess the relative contributions of fat and lean tissue to the deposited mass before migration (Lindström and Piersma 1993). However, a plot of lean mass on fat mass (Fig. 3) suggested that some lean mass may have been deposited along with fat, though there was an unusually high degree of scatter in the data. In the heavy birds, there was considerably larger variation in lean tissue mass than in fat mass, which might possibly be sex related (Fig. 3, filled symbols are the females, which tended to have more lean mass).

The two groups of fasting birds were clearly separated (Fig. 3), the postmigration group being higher in lean tissue but lower in fat than the experimentally fasted group (Tables 2, 3). Lines of equal composition and equal body mass are also plotted in Figure 3, and there is no indication of a break point in body composition (when stores are zero, below which structural mass is catabolized; van der Meer and Piersma 1994).

The female from China that did appear to be nutrient

stressed (Fig. 2, *filled circle*) had a total of only 4.1 g of fat in her body. It was lower in fat in virtually all organs than all the other nine birds from China and had exhausted the discrete fat deposits (0.2 g). Total lean tissue in this bird was 82% of the value of the other postmigration birds. Most of the difference was found in the exercise organs, which were only 53% of the mass of the other birds, while its nutritional organs were maintained at 96%. Compared with the predeparture group, its exercise and nutritional organs were 43% and 66%, respectively, with total lean tissue also being only 66%.

Across the six females analyzed (three before migration and three after migration), body composition changes were more extreme than in the male birds. We are unable to assess whether this is a biological reality or a sampling artifact. Predeparture females ($n = 3$) were heavier than males ($n = 7$; 245 vs. 228 g, $P = 0.0473$; statistics from two-sample randomization tests) and slightly fatter (7.7%, $P = 0.1392$) when caught and larger in most lean organ masses, especially the liver (42%, $P = 0.0001$), kidneys (23%, $P = 0.0001$), salt glands (45%, $P = 0.0001$), and stomach fresh mass (49%, $P = 0.0001$). Total nutritional organ mass was higher (33%, $P = 0.0001$), as were the exercise organs (11%, $P = 0.0451$), mainly due to the flight muscles (11%, $P = 0.046$) and leg muscles (13%, $P = 0.0243$). After migration, females tended to be equivalent to or slightly lower than males in most organs but larger in salt glands (11%), fresh heart (21%), and stomach (23%) masses. Randomization tests, however, detected no significant differences between the sexes after migration in any organ masses, total lean mass, total exercise, or total nutritional organ masses ($0.13 < P < 0.93$).

Discussion

Energy Used and Relative Protein Contribution

By comparing the nutrient status of birds before and after flight, the energy used and relative protein contribution (percentage of energy derived from protein [RPC]) can be determined. In this study, birds were collected immediately after a fast (captive) or probably within a day or so of arrival after migration (China), giving good postfast data. Predeparture birds were not all at their probable departure masses (and migratory departures were not seen until 6 d later). We approached this problem by first calculating the apparent RPC value based on our samples, assuming the RPC to be the same for any tissues built up

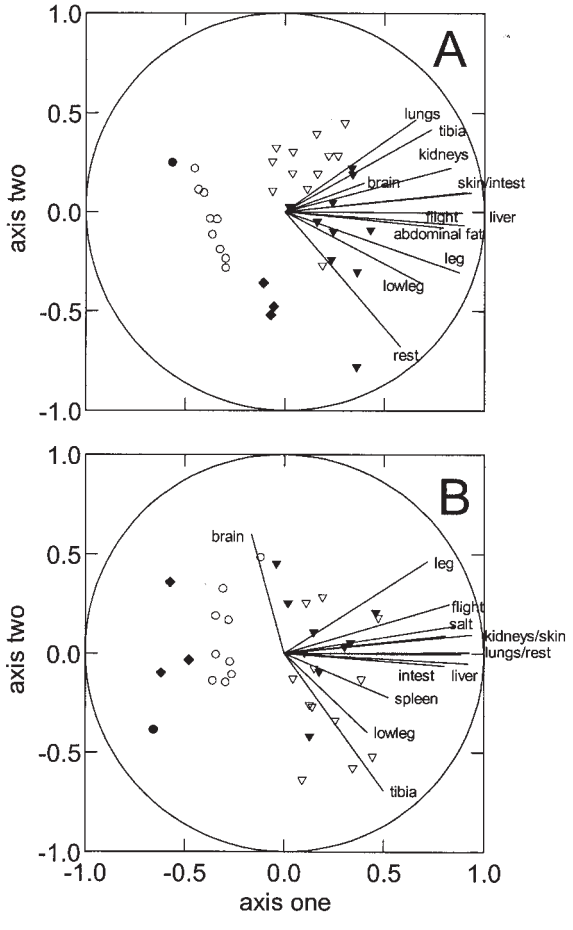

Figure 2. Relationships between organ fat (*A*) and lean (*B*) tissue mass between individual great knots, based on a biplot of a principal components analysis. The axes represent the first two principal components of the size-corrected data for all birds. Each plot shows a vector for the relationship between each organ and the first two principal components. The vectors are the principal component loadings and provide a simple visual interpretation of the axes. The length of the vector indicates the reliability of the approximation; this is the R^2 value, or how much of the variance in an organ is explained by the two axes. The angle between two vectors gives the degree of correlation between them. Adjacent vectors are highly correlated with each other, and the length of the vector indicates the strength of the relationship. Orthogonal (right angled) vectors are uncorrelated, and vectors pointing in opposite directions are negatively correlated. In both plots, the vectors indicate that axis 1 relates mainly to overall organ sizes; axis 2 provides more discrimination between organs. For each bird, the value of the second principal component is plotted against the value of the first principal component, with different symbols for the different groups: *open triangles*, fueling; *filled triangles*, predeparture; *open circles*, postmigration; *filled circle*, an apparently nutrient-stressed female postmigration; *filled diamonds*, fasted. The biplot thus shows groupings among individuals of different masses and migratory states, as well as the similarity of changes of individual organs. The total variance explained by the two principal component axes in the fat data (*A*) was 71.1% (61.3% by axis 1 and 9.8% by axis 2) and in the lean tissue data (*B*) was 66.8% (56.3% by axis 1 and 10.5% by axis 2). Stomach and heart muscles are not included because the fat and lean tissues were not directly determined. Skin/intest (*A*) and kidneys/skin and lungs/rest (*B*) have overlapping or partially overlapping vectors. Flight refers to flight muscles, intest to the intestine, leg to leg muscles, lowleg to the lower leg, and salt to the salt glands. Component loadings are provided in the appendix.

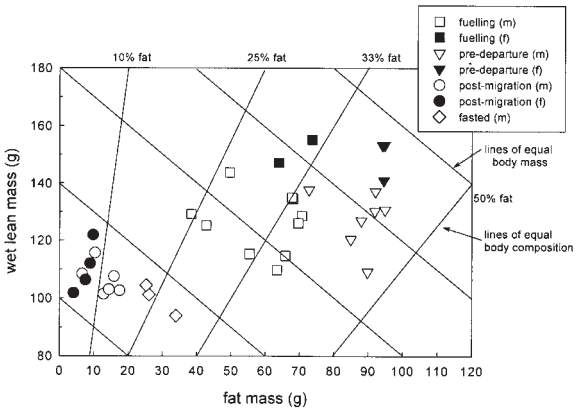

Figure 3. Lean mass plotted against fat mass in great knots during periods of tissue buildup and breakdown. The plot includes females as well as males and birds killed earlier in the migratory fueling period than the premigration sample. The lines sloping down from the *Y*-axis are lines of equal body mass. The lines sloping up from the *X*-axis are lines of equal body composition. Filled symbols are females; open symbols are male.

and broken down above the body masses sampled (mean 239.4 g). We then simulated the RPC that would result if birds departed 10 g heavier at 250 g, the additional tissue being composed entirely of fat or (improbably) protein. With energy densities of 39.6 kJ g^{-1} for dry fat and 17.8 kJ g^{-1} for dry lean tissue (Jenni and Jenni-Eiermann 1998), the RPC estimates were 4.0% (239.4 g sample), 3.5% (250 g fat), and 5.6% (250 g lean; Table 4). In case these results were biased by the inclusion of both sexes (the females having higher lean masses; Fig. 3), we made the same calculations for the males only but departing at 226.7 and 245 g. The results are similar: a basic RPC of 3.5% at 226.7 g, and 2.8%–6.5% at 245 g.

For the fasting birds killed for body composition, while their initial mass was very close to that of other males collected on March 21 (227.6 vs. 226.7 g), the feeding while transported to The Netherlands is a complication. This feeding had little effect on the rate of body mass loss (Fig. 1*B*), and the three killed birds lost 40, 46, and 53 g between capture and the start of the actual fast. The feeding will have supplemented the energy derived from stored tissues to some degree, so the RPC value therefore represents the net change in body tissues, rather than the contribution to the real energy turnover (which will have been higher than calculated). Under these conditions, the RPC for the fasted birds was 6.8% (Table 4). As the composition of the food eaten after capture will be lower in fat and higher in protein than the relative nutrient stores in the body, the RPC for the true energy turnover is probably higher. The mean respiratory quotient for the experimentally fasted birds during nighttime respirometry (P. F. Battley, A. Dekinga, M. W. Dietz, T. Piersma, S. Tang, and K. Hulsman, unpublished manuscript) was 0.706 (range 0.64–0.77, $n = 25$ measurements on eight birds), confirming that fat was the primary fuel used.

Rates of Energy Turnover

The fasting birds used less energy than the migrating ones for a similar change in body mass (Table 4). Because the real energy turnover of the migrants will be higher than the 3,229.3 kJ calculated for the first case in Table 4, we used a figure of 3,500 kJ (which would result from the use of 84.9 g fat and 7.9 g protein at an RPC of 4.0%).

The duration of the flight from Australia to China is thought to be about 4 d, while the mean length of fast in the three experimental birds was 14 d (13, 13, and 16 d, respectively). The estimated energy turnover rates are 875 kJ d^{-1} for the migratory flight (3,500 kJ per 4 d) and 181 kJ d^{-1} for the fast (2,537 kJ per 14 d). Migrating birds expended energy at 4.8 times the rate of fasting birds. These costs can also be expressed in relation to basal metabolic rate (BMR), which was 1.85 W in five fueling and premigratory great knots (Battley 2000*b*). During migration, the birds had an energy turnover of 10.1 W, so the estimated flight cost was 5.5 times BMR. This may be an underestimate of the real flight cost, as BMR declines during migration (Battley et al. 2000*a*). If the mean BMR was 1.47 W (midpoint between averages of pre- and postmigration measurements), the flight cost would be 6.9 times BMR.

The three fasting birds killed for body composition analyses had a mean BMR during the fast of 1.28 W (P. F. Battley, unpublished data) and an energy turnover of 2.09 W, resulting in an existence cost of 1.6 times BMR.

Flight Costs during Migration: A Consistency Analysis

The estimated flight costs (5.5–6.9 × BMR) are low compared to predicted costs based on literature. Masman and Klaassen's (1987) Equation (6) and Norberg's (1996) Equations (7.35) and (7.36) estimate flight costs between 12 and 14.5 W (6.5–9.9 × BMR). The calculated flight costs would be too low if the total energy turnover was underestimated or if the flight duration was overestimated (by not accounting for wind assistance; Tulp et al. 1994). Accordingly, we explored how varying the assumptions of flight duration and energy used affects the proposed flight cost. In Figure 4*A*, the flight costs (presented as kJ h^{-1}) are plotted for different flight durations. Flight durations are also shown as flight speeds on the right-hand *Y*-axis (assuming a 5,420 km flight). Total energy expenditure varies in 10% increments each side of 3,500 kJ, so the +20% line shows the relationship for 4,200 kJ and the −20% line the equivalent for 2,800 kJ. The box encloses flight speeds of 60–85 km h^{-1}, which covers expected flight speeds with no to moderate wind assistance, and the shaded portion encompasses the range of values from Masman and Klaassen (1987) and Norberg (1996), calculated above (expressed this time as kJ h^{-1}). The

Table 4: Estimates of relative energy contribution from fat and lean tissue in great knots during flight or fasting

	Initial Mass (g)			Final Mass (g)			Mass Change (g)		Energy Expenditure (kJ)			
	Body	Fat	Lean Dry	Body	Fat	Lean Dry	Fat	Lean	Fat	Lean	Total	RPC (%)
Flight:												
1	239.5	89.9	37.4	126.0	11.6	30.1	78.3	7.2	3,100.7	128.6	3,229.2	4.0
2	250	100.3	37.4	126.0	11.6	30.1	88.8	7.2	3,515.8	128.6	3,644.4	3.5
3	250	89.9	40.6	126.0	11.6	30.1	78.3	10.5	3,100.7	186.4	3,287.1	5.7
Fasting	227.5	88.1	36.2	129.3	28.4	26.5	59.7	9.7	2,364.1	171.8	2,535.8	6.8

Note. Three calculations for birds during flight are made. The first is for an initial mass of 239.5 g, which will underestimate true energy turnover (see text). The following two calculations are for birds leaving at 250 g. In 2, the difference in mass between the original measurement and the estimate at departure is entirely fat; in 3, the difference is entirely protein (assuming water content of 69%). Note that the value for the fasting birds is not a true fast; food was provided for the first 5 d of the fast, though body mass continued to be lost during this period (see text). Assumed energy densities of fuel are 39.6 kJ g^{-1} for dry fat and 17.8 kJ g^{-1} for dry lean tissue (Jenni and Jenni-Eiermann 1998). Masses are corrected for body size and water loss during capture where appropriate. RPC = relative protein contribution. Values are rounded to one decimal place.

values enclosed in the box overlap substantially with the predicted flight costs, although they tend to be lower.

Another check on how realistic our values are can be made by plotting the total amount of lean tissue used against RPC values for different energy levels (Fig. 4*B*). On the *X*-axis, the amount of protein that would be broken down for a given RPC value and total energy used is plotted. Total energy used is varied in the same way as in Figure 4*A*. For example, the thick central line shows how much protein would be catabolized if 3,500 kJ of energy were used under different RPC values. The box encloses what we feel to be realistic levels of protein catabolism (6–10-g lean dry mass). It is apparent that higher levels of energy turnover would result in large amounts of lean tissues being broken down, except at the very lowest RPC values. It is also clear that the protein contribution must be low (<7%) in order for only 6–10 g of protein to be used. In these hypothetical cases, fat consumption ranged from 66 to 101 g for values covering 6–10-g lean tissue use, which, after adding residual fat left after migration, would mean a departure fat mass of 89–114 g. The heaviest female great knot analyzed had only 97 g of fat. This implies that we have not substantially underestimated the energy turnover in these birds.

How Legitimate Is an Experimental Fast to Simulate Migratory Changes?

A problem with using fasting to simulate migration is whether the fast is long enough to introduce new factors into the changes in body composition. Five recent studies (Klaassen and Biebach 1994; Hume and Biebach 1996; Biebach 1998; Karasov and Pinshow 1998, 2000) all dealt with small birds (garden warbler, *Sylvia borin*, and blackcap, *Sylvia atricapilla*), which were fasted for relatively short periods, varying from 1.5 to 6 d (four studies fasted birds for a fixed period; one fasted birds down to a target mass). Our knots fasted for 13, 13, and 16 d, respectively, and while they reached a similar body mass to the migrated birds, the total energy expended was lower. Karasov and Pinshow (1998) noted that while the total energy metabolized might be similar between a migrating and fasting bird, the breakdown of body protein might be quite different. Differences in fasting duration and total energy metabolized could have affected body composition. While the fasted birds may give insight into the processes behind the observed organ changes in the migrating birds, the long duration of the fast may have obscured some of the more meaningful comparisons. We predicted that the exercise muscles would be smaller in the flown birds than in the fasted birds, but this was not found, a result that could easily be due to the long fast.

A striking difference that is apparent between great knots and the garden warbler studies is in the time course of changes in the nutritional organs. Hume and Biebach (1996) documented reductions of 50% in digestive tract and 63% in small intestine dry masses in a fast of only 2 d. Great knots showed a reduction in intestine lean tissue of 43% but over 2 wk (Table 3). This could be a result of the higher mass-specific metabolic rate of the warblers or reflect an underlying difference in rates of cell turnover in these organs related to dietary habits. A diet-induced reduction in stomach size of 40% in red knots in captivity took about 10 d (Piersma et al. 1999*a*).

The Three Phases of Fasting

Are the three phases during fasting, which have been described from essentially inactive animals (Cherel et al. 1988), found in actively exercising animals such as migrating birds? Jenni et al. (2000) recently showed that passerines and doves arriving in Italy from North Africa with low fat scores also had high uric acid levels. This indicates that these birds had entered the equivalent of phase 3, with a suggested threshold fat level (Cherel et al. 1992) of 4%–5% of body mass (Jenni et al. 2000). Because we do not have plasma-based assessments of relative rates of tissue catabolism for our great knots (Cherel and Le Maho

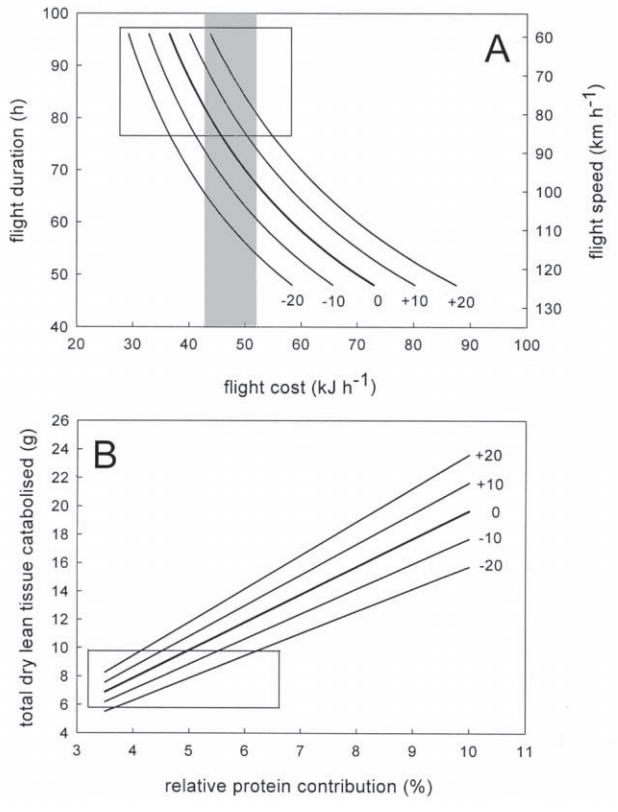

Figure 4. *A*, How flight duration and total energy expended affect the calculated flight cost for great knots flying from Australia to China. Flight costs are calculated as total energy used divided by time (flight duration, on the *Y*-axis). The thick central line shows the relationship if 3,500 kJ of energy is used (see text). In case we under- or overestimated the total energy consumed, this is varied in 10% increments, where a positive value means more energy was used than we assumed. The box encloses values of 60–85 km h^{-1} (right-hand *Y*-axis), and the shaded column shows flight cost values calculated from Masman and Klaassen (1987) and Norberg (1996). *B*, How total protein breakdown differs according to total energy expended for different relative protein contributions (RPC). Using the same variation in total energy as in *A*, lean tissue used is plotted against the RPC value. The box encloses catabolism of 6–10 g of total lean dry tissue.

1985; Groscolas 1986; Boismenu et al. 1992; Lindgard et al. 1992; Handrich et al. 1993), we can work only with rates of body mass loss in captivity and body composition differences. Comparable body composition data on other fasting animals are scarce (Le Maho et al. 1981; Parker and Holm 1990; Cherel et al. 1995).

In the fasting great knots, rates of body mass loss were virtually constant within individuals for most of the fast, and there was no evidence for an accelerated mass loss toward the end of the fast. Body mass (Fig. 1*B*) and daily mass-specific changes in body mass (not shown) showed no increase before the end of the fast. This suggests that birds had not reached phase 3. The fasted knots retained considerable fat stores while having used a high amount of lean tissue during the fast, whereas the migrated birds had used most of their fat stores but had depleted less lean tissue than the experimentally fasted birds. Weber and Piersma (1996) also found that a wild red knot from the breeding grounds had less fat tissue and more lean tissue than captive red knots of equivalent mass at the end of a voluntary fast. This suggests that there is no set fat threshold for increased protein catabolism in our fasted and flown great knots. There was no evidence of a break point in the plot of lean mass on fat mass that would be expected if birds had exhausted their strategically deposited nutrient stores (van der Meer and Piersma 1994). These findings suggest that (*a*) the fasted birds did not use fat and protein in the same way as other studied fasting birds and (*b*) most of the migrated birds did not reach phase 3 of a fast.

Other migrating birds apparently do enter phase 3 (Jenni et al. 2000). One great knot probably had done so—the female with extremely reduced exercise organs. In contrast to the birds in Jenni et al.'s (2000) study, this great knot had nutritional organs of equivalent size to the other birds (the Italian birds in phase 3 had especially reduced digestive organs). The data in Jenni et al. (2000) also suggest that perhaps 30%–40% of the long-distance migrants that arrived with a wide range of fat stores showed elevated uric acid levels (based on their Fig. 2). One of 10 great knots caught in China in this study had broken down an especially large amount of lean tissue, but without knowing the relationship between plasma metabolite levels and body composition, we cannot say how the other nine birds would compare to the passerine study.

In fasting barn owls, *Tyto alba*, bone marrow fat stays constant until phase 3, when it is mobilized from all parts of the skeleton (Thouzeau et al. 1997). In the migrated great knots, the fat content of the tibia and lower leg were lower than in predeparture birds, suggesting that lipids may be mobilized from parts of the skeleton without the other deposits being exhausted. In absolute terms, the amount of fat catabolized in these bones is trivial, but it indicates that the metabolic characteristics of flying birds may differ from those of inactive fasting birds.

The RPC estimates for the migrating birds, even with some uncertainty about the true value, suggest that the migrating birds were extremely economical at conserving protein. An RPC of 4% is among the lowest recorded in birds (Jenni and Jenni-Eiermann 1998). Jenni and Jenni-Eiermann (1998) showed that the RPC decreases with initial fat content up to around 25%–35% fat, above which there is little further decrease in the RPC of 5%. Our data from the migrated birds agree with this; the fat content of almost 40% being higher than any birds in their analysis. However, Jenni and Jenni-Eiermann reported similar RPC values for the few cases in the literature in which there was a comparison between low- and high-energy-turnover fasts. In our study, the flown birds had an energy

turnover of almost five times and an RPC value of less than two-thirds that of the fasted birds. (The RPC of the fasted birds was, however, still similar to other studies [Jenni and Jenni-Eiermann 1998].)

Rather than this difference relating to relative differences in the likely roles of amino acids during flight or fasting (to maintain the supply of citric acid–cycle intermediates and substrates for gluconeogenesis; Jenni and Jenni-Eiermann 1998), the higher RPC value for the fasting birds is probably a consequence of the unnaturally long fast, although protein turnover tends to decrease during starvation in a range of animals (Hawkins 1991). In buzzards, *Buteo buteo*, that were starved for 13 d, however, no protein conservation was apparent, with levels of urea and uric acid increasing steadily through the fast (García-Rodríguez et al. 1987).

So, is flight simply a high-energy fast? In terms of body composition, both flown and fasting birds drew lean tissue from virtually all organs in the body, though the fasted birds used more lean tissue, especially from the nutritional organs. Both groups also used fat from similar deposits and organs. There were no organs or nutrient deposits that unambiguously distinguished between the flown and fasted birds, apart from perhaps liver and bone fat. What differed was the extent to which the organs or deposits were broken down. Our results were certainly affected by the long duration of the experimental fast, which was itself a consequence of the size of the birds and the amount of fuel stored. Smaller passerines with higher mass-specific metabolic rates might well show more comparable changes in composition over shorter time frames. The low RPC values calculated confirm that fat migrating birds conserve protein effectively, as is also found in low-energy fasting animals. It remains to be seen whether the metabolic patterns identified in inactive fasting animals are also found in migrating birds. The indication from Jenni et al. (2000) based on plasma metabolites is that they do. Our comparison of body composition analyses suggests that some details differ in extreme long-haul migrants. Wind tunnel experiments are the ideal tool for investigating such questions (Lindström et al. 1999).

The apparent contradiction of an extremely low RPC value yet substantial organ reductions in the migrated birds reflects the large magnitude of the enterprise. This flight from Australia to China is one of the longest documented in the world, and total energy turnover is high. Piersma (1998) developed the idea that the size of organs at departure represents evolutionary compromises between the functional roles of organs at different stages of the migration. If our interpretation that lean tissue may be catabolized from almost the entire suite of organs in the body is correct, then the organ masses at takeoff may be critical. In particular, failure to deposit enough lean tissue in the flight muscles, skin, and skeletal muscle (the main sources of protein in the flown birds) could lead to excessive breakdown from other organs with current or upcoming strategic importance.

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Appendix

Table A1: Component loadings of principal component analyses of organ fat and lean masses

	Fat Tissue		Lean Tissue	
Organ	Component 1	Component 2	Component 1	Component 2
Flight muscles	.890	−.004	.835	.056
Leg muscles	.875	−.305	.756	−.126
Skin	.934	.098	.826	−.034
Abdominal fat	.899	−.069	…	…
Intestine	.912	.100	.918	.081
Liver	.796	−.079	.923	.136
Kidneys	.835	.221	.932	.186
Lungs	.658	.466	.782	.220
Brain	.398	.146	−.160	.020
Rest	.581	−.678	.871	.122
Tibia	.737	.417	.137	.828
Lower leg	.691	.361	.411	−.801
Salt glands	…	…	.874	−.128
Spleen	…	…	−.260	.932

Note. Shown visually in Figure 2.

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